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THE INFLUENCE OF AFTER-CROP PLANT MULCH AND ONION CULTIVATION ON MICROORGANISM COMPOSITION IN SOIL

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Abstract. The aim of the research was to determine the quantitative and qualitative composition of the communities of fungi and bacteria formed in the soil under the influence of onion cultivation, with consideration to various options of conserving cultivation, using spring rye and common vetch as after-crop cover plants. Moreover, in the laboratory tests, the occurrence of microorganisms characterizing by an antagonistic influence on pathogenic fungi with a facultative parasiting was established. As a result of the laboratory microbiological analysis it was found out that particular soil samples taken from under the cultivation of onion differed with the qualitative and quantitative composition of microorganisms. Spring rye stimulated the growth and development of microorganisms, especially antagonistic ones (*Bacillus* spp., *Pseudomonas* spp., *Gliocladium* spp., *Trichoderma* spp.), and common vetch caused an increase of the number of cfu of pathogens (*Alternaria alternata, Fusarium culmorum, F. oxysporum, F. solani, Penicillium* spp., *Pythium irregulare*).

Key words: onion, antagonistic bacteria, antagonistic fungi, pathogenic fungi, conservation tillage, spring rye, common vetch

INTRODUCTION

Present in soil are communities of bacteria populations and fungi which have different influence on each other, as well as on plants. Particular cultivated plant species have a significant influence, through their root exudate, on the growth and development of the saprobiotic and pathogenic microorganism populations occurring in and outside of the rhizosphere [Batalin 1962, Schroth and Hildebrand 1964, Rovira 1965, Huber and Watson 1970, Funck-Jensen and Hockenhull 1984]. Root exudate is a rich source of

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compounds that stimulate (sugars, acidic amino acids) and inhibited (phenol compounds, aromatic and alkine amino acids) the development of pathogenic fungi [Sytnik et al. 1977, Milczak and Piotrowski 1980, Piotrowski and Milczak 1982, Funck-Jensen and Hockenhull 1984, Pięta 1988].

Special attention should be paid to the stimulating effect of the root exudate on the growth and development of antagonistic microorganisms which counter the soil-borne phytopathogens.

Many researchers distinguish between microorganisms which are unfavorable (deleterious rhizosphere microorganisms – DRMO) and favorable to a plant (plant growth promoting rhizobacteria – PGPR) [Rovira 1969, Curl 1982, Funck-Jensen and Hockenhull 1984, Pięta and Patkowska 2001]. Moreover, each plant species, through crop residues or the green fertilizer, modifies the composition of the microorganism population.

The aim of the research was to determine the quantitative and qualitative composition of the communities of fungi and bacteria formed in the soil under the influence of onion cultivation, with consideration to various options of conserving cultivation, using spring rye and common vetch as after-crop cover plants. Also, on the basis of laboratory tests, established was the occurrence of microorganisms characterized by an antagonistic influence on pathogenic fungi with a facultative parasitism. Pathogenic activity of the fungi for the onion, often isolated from the soil, was also determined.

MATERIAL AND METHODS

1. Microorganisms communities in the soil from onion cultivation using spring rye and common vetch as cover plants

Field experiments were carried out in years 2004–2006 on soil derived from medium silt clay [Pięta and Kęsik 2007]. The experiment plant was onion (*Allium cepa* var. *cepa* Helm.) of a 'Wolska' cv. In the research considered were the mulching after-crop palnts: spring rye and common vetch, as well as differentiated land cultivation. The after-crop cover plants were sowed in summer (VII/VIII) in the years preceding the cultivation of onion. Prior to the winter season, these plants had yielded a great crop of green mass, which constituted a natural plant mulch on the surface of the soil and which was mixed with the soil in a various ways: 1) leaving the mulch on the surface of the land until spring and direct sowing of onion without prior soil cultivation (no tillage); 2) rotary ploughing prior to winter and during spring plus pre-sowing additional cultivation measures; 3) leaving the mulch on the surface of the land over the winter, rotary ploughing during the spring plus pre-sowing additional cultivation measures. The control was the traditional cultivation without the mulching plants including deep pre-winter tillage and a set of spring pre-sowing cultivations.

The research subjects were soil samples taken yearly in the second half of June on the depth of 5–6 cm.

These soil samples were used to prepare water solutions of the soil solution using the methods described by Martyniuk et al. [1991] and Pięta and Patkowska [1997]. In order to isolate the colonies of bacteria and fungi, an agar grounds were used, such as the malt medium, Martin, PDA, Nutrient agar, Tryptic soy agar, and Pseudomonas agar F. In order to obtain all bacteria colonies, a diluted soil solution was used of 10^{-5} to 10^{-7}, for *Bacillus* spp. A diluted solution of 10^{-4} to 10^{-6} was used, while for *Pseudomonas* spp. a diluted solution of 10^{-2} to 10^{-4}. For determining the occurrence of fungi species in the investigated samples used were diluted soil solutions of 10^{-2} to 10^{-4}. Frequently occurring fungi were classified into particular species using keys, monographs, and papers.

2. Determining the antagonistic influence of saprobionts in relation to soil-borne pathogenic fungi

The research subjects were the saprobiontic bacteria and fungi isolated from the analyzed soil samples. The tests were carried out in laboratory conditions. The antagonistic influence of the isolates of *Bacillus* spp., *Pseudomonas* spp., *Trichoderma hamatum*, *T. harzianum*, T. *koningii* and *T. viride* was measured in relation to such pathogenic fungi as *Alternaria alternata, Botrytis cinerea, Fusarium culmorum, F. oxysporum, F. solani, Phoma exigua* var. *exigua, Pythium irregulare, Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Methods described by Huang [1978] and Dos Santos and Dhingra [1982] were used for measuring the influence of saprobiontic fungi in relation to phytopathogens. For measuring the antagonistic influence of the bacteria, used were the methods described by Martyniuk et al. [1991] and Pięta [1993]. In the final stage of the experiment, a summary antagonistic effect for microorganism communities occurring in individual soil samples was calculated.

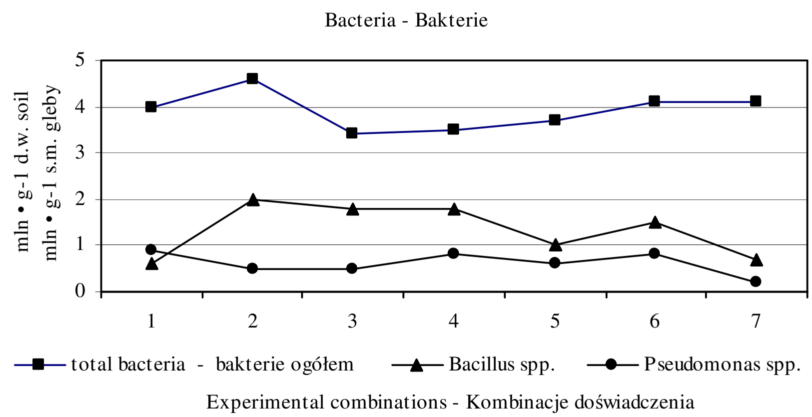
3. Harmful influence of soil-borne fungi on onion

In the growing chamber experiments, in order to determine the pathogenic character of the fungi isolated from the investigated soil samples, used were 15 isolates each, of such species as *Fusarium culmorum, F. oxysporum, F. solani, Penicillium verrucosum* var. *cyclopium, P. verrucosum* var. *verrucosum* and *Pythium irregulare*. Garden soil with corn middling was sterilized (2 hours in the temperature of 121°C and pressure of 1 atmosphere) and inoculated with the individual species of the mentioned fungi. Three weeks later, the soil streaked with the fungi was used to fill the pots, in which the onion of the 'Wolska' cv. was sowed. Each pot was seeded with 100 seed of the 'Wolska' cv. onion. Each isolate of the pathogen was assigned 4 pots (4 repetitions). 3–4 weeks after the experiment had been started, the number of grown plants was counted and their health was determined.

RESULTS

1. Microorganism communities in the soil from onion cultivation with the use of spring rye and common vetch as cover plants

As a result of the microbiological analysis of the soil, it was established that the greatest number of total bacteria was obtained in the soil where the spring rye mulch had been used and rotary ploughing was performed in the spring (fig. 1). A high total number of colony form unites (cfu) bacteria was also obtained in the soil samples taken



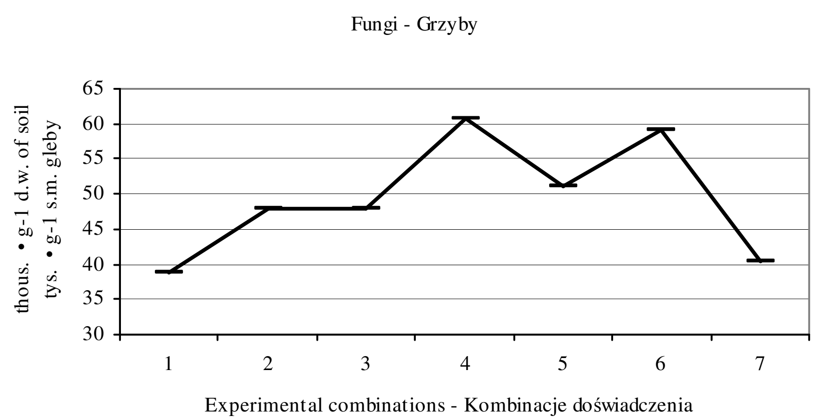
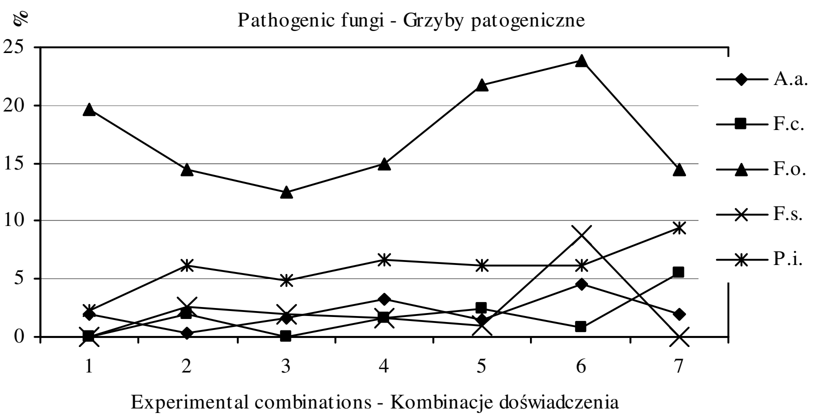
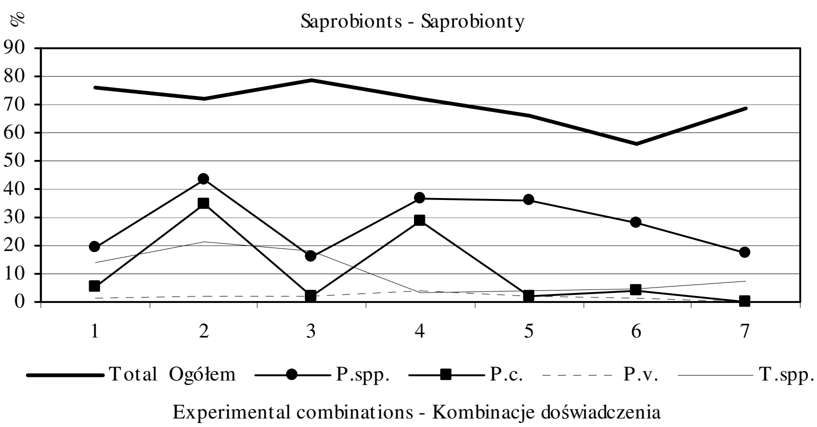


Fig. 1. Mean of number cfu bacteria and fungi isolated from the soil particular experiments combination: 1 – mulch of spring rye + direct onion sowing, 2 – mulch of spring rye + disc harrowing in spring and onion sowing, 3 – mulch of spring rye + disc harrowing in autumn and spring and onion sowing, 4 – mulch of common veltch + direct onion sowing, 5 – mulch of spring rye + disc harrowing in spring and onion sowing, 6 – mulch of spring rye + disc harrowing in autumn and spring and onion sowing, 7 – traditional cultivation

Rys. 1. Średnia liczba jtk bakterii i grzybów wyizolowanych z gleby poszczególnych kombinacji doświadczenia: 1 – mulcz żyta jarego + bezpośredni siew cebuli, 2 – mulcz żyta jarego + talerzowanie wiosną i siew cebuli, 3 – mulcz żyta jarego + talerzowanie jesienią i wiosną i siew cebuli, 4 – mulcz wyki siewnej + bezpośredni siew cebuli, 5 – mulcz wyki siewnej + talerzowanie wiosną i siew cebuli, 6 –- mulcz wyki siewnej + talerzowanie jesienią i wiosną i siew cebuli, 7 – uprawa tradycyjna



A.a. – *A. alternata*, F.c. – *F. culmorum*, F.o. – *F. oxysporum*, F.s. – *F. solani,* P.i. – *P. irregulare*



Total Ogółem – Total saprobionts saprobionty ogółem, P. spp. – *Penicillium* spp., P.c. – *P. verrucosum* var. *cyclopium*, P.v. – *P. verrucosum* var. *verrucosum*, T. spp. – *Trichoderma* spp.

Fig. 2. Participation pathogenic fungi and saprobiotic in soil under onion cultivation particular experiments combination: 1 – mulch of spring rye + direct onion sowing, 2 – mulch of spring rye + disc harrowing in spring and onion sowing, 3 – mulch of spring rye + disc harrowing in autumn and spring and onion sowing, 4 – mulch of common veltch + direct onion sowing, 5 – mulch of spring rye + disc harrowing in spring and onion sowing, 6 – mulch of spring rye + disc harrowing in autumn and spring and onion sowing, 7 – traditional cultivation

Rys. 2. Udział grzybów patogenicznych i saprobiontów w glebie spod uprawy cebuli w różnych kombinacjach doświadczenia (średnia z lat 2004–2006): 1 – mulcz żyta jarego + bezpośredni siew cebuli, 2 – mulcz żyta jarego + talerzowanie wiosną i siew cebuli, 3 – mulcz żyta jarego + talerzowanie jesienią i wiosną i siew cebuli, 4 – mulcz wyki siewnej + bezpośredni siew cebuli, 5 – mulcz wyki siewnej + talerzowanie wiosną i siew cebuli, 6 – mulcz wyki siewnej + talerzowanie jesienią i wiosną i siew cebuli, 7 – uprawa tradycyjna from the combination where the spring rye mulch with direct onion sowing, as well as the mulch of common vetch with rotary ploughing in the autumn and spring had been used, as well as in the traditional cultivation (fig. 1). The smallest total number of cfu bacteria was found in the soil sample where the spring rye mulch had been rotary-ploughed in the spring and in the autumn. The greatest number of cfu *Bacillus* spp. was observed in the soil taken from the combination where the spring rye mulch had been rotary ploughed in the spring with direct sowing of onion. The least bacteria were found in the soil taken from the combination where the spring rye mulch had been left on the field surface and in the traditional cultivation. In the case of *Pseudomonas* spp., the greatest number of cfu bacteria was obtained when the spring rye mulch and the common vetch mulch were left on the soil surface and onion was direct sowed. Moreover, the number of cfu *Pseudomonas* spp. was high in the soil where the common vetch mulch had been rotary ploughed in the autumn and in the spring (fig. 1). The smallest total number of cfu fungi was found in the samples taken from the combination where the rye mulch had been left on the soil surface and in the traditional cultivation (fig. 1). Regardless of the treatment of the common vetch mulch, the samples taken from these combinations contained the most fungi colonies (fig. 1).

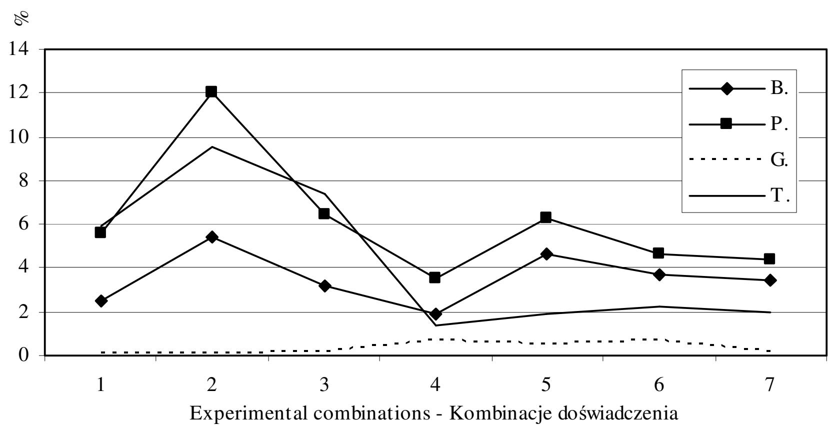
Among the fungi isolated from the investigated soil samples, the most isolates classified as pathogens, especially *Fusarium* spp., were found after using the common vetch as after-crop (fig. 2). The *Fusarium* genus was represented by *F. culmorum*, *F. oxysporum* and *F. solani*.

2. Determining the antagonistic influence of saprobionts in relation tosoil-borne pathogenic fungi

The laboratory tests showed various numbers of the antagonistic bacteria and fungi in the particular soil samples. Much higher numbers of such microorganisms were found in the soil from onion cultivation with the use of spring rye as the cover plant, than in that where common vetch had been used (fig. 3). The method of mulch treatment, both for spring rye and common vetch, had an impact on the quantitative and qualitative composition of the antagonistic microorganisms. Regardless of the specie of the cover plant, the rotary ploughing in the spring increased the antagonists in the soil. The microbiological analysis showed that rotary ploughing of the mulch of the cover plants in the fall and in the spring somewhat decreased the number of cfu antagonists. The mulch left on the soil surface had little effect on the growth and development on the antagonistic bacteria (*Bacillus* spp., *Pseudomonas* spp.) and fungi (*Gliocladium* spp., *Trichoderma* spp.) (fig. 3). In the case of the combinations with spring rye, *Pseudomonas* spp. and *Trichoderma* spp. represented by *T. hamatum*, *T. harzianum*, *T. koningii* and *T. viride* dominated.

3. Harmful effect of soil-borne fungi on onion

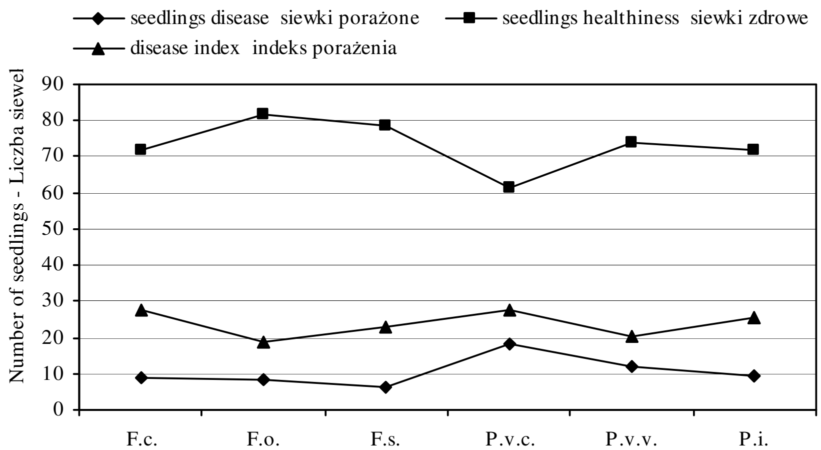
The fungi species used in the growing chamber experiment proved pathogenic for the onion seedlings. Individual fungi species used for the artificial infection, such as *Fusarium culmorum, F. oxysporum, F. solani, Penicillium verrucosum* var. *cyclopium, P. verrucosum* var. *verrucosum* and *Pythium irregulare* were characterized by differentiated pathogenicity towards the onion (fig. 4). On average, the least seedlings were found in the combination with *Fusarium culmorum, Penicillium verrucosum* var. *cyclopium* and *P.verrucosum* var. *verrucosum*, whereas the greatest number of infected plants (those with necrotic stains on roots and leaves) were found in the combination with *P. verrucosum* var. *cyclopium* i *P. verrucosum* var. *verrucosum*. The highest infection index was calculated in the case of the seedlings from the combination with *F. culmorum* i *P. verrucosum* var. *cyclopium* (fig. 4). Among the pathogenic fungi, the smallest harmful effect was observed for *F. oxysporum* i *F. solani* (fig. 4).



B. – *Bacillus* spp., P. – *Pseudomonas* spp., G. – *Gliocladium* spp., T. – *Trichoderma* spp.

Fig. 3. Participation antagonistic bacteria and fungi in the soil particular experimental combination (mean 2004–2006): 1 – mulch of spring rye + direct onion sowing, 2 – mulch of spring rye + disc harrowing in spring and onion sowing, 3 – mulch of spring rye + disc harrowing in autumn and spring and onion sowing, 4 – mulch of common veltch + direct onion sowing, 5 – mulch of spring rye + disc harrowing in spring and onion sowing, 6 – mulch of spring rye + disc harrowing in autumn and spring and onion sowing, 7 – traditional cultivation

Rys. 3. Udział antagonistycznych bakterii i grzybów w glebie poszczególnych kombinacji doświadczenia (średnia z lat 2004–2006): 1 – mulcz żyta jarego + bezpośredni siew cebuli, 2 – mulcz żyta jarego + talerzowanie wiosną i siew cebuli, 3 – mulcz żyta jarego + talerzowanie jesienią i wiosną i siew cebuli, 4 – mulcz wyki siewnej + bezpośredni siew cebuli, 5 – mulcz wyki siewnej + talerzowanie wiosną i siew cebuli, 6 – mulcz wyki siewnej + talerzowanie jesienią i wiosną i siew cebuli, 7 – uprawa tradycyjna



F.c. – *Fusarium culmorum*, F.o. – *F. oxysporum*, F.s. – *F. solani*, P.v.c. – *Penicillium verrucosum* var. cyclopium, P.v.v. – *P. verrucosum* var. *verrucosum*, P.i. – *Pythium irregulare*

Fig. 4. Influence of pathogenic fungi on number and healthiness of seedlings onion in the growing chamber experiments

Rys. 4. Wpływ grzybów występujących w glebie na liczebność i zdrowotność siewek cebuli w doświadczeniu fitotronowym

DISCUSSION

The microbiological analysis revealed a differentiated quantitative and qualitative composition of the microorganism communities in the soil from onion cultivation with the use of spring rye and common vetch as after-crop cover plants. Therefore, the selection of the after-crop plant may have a great impact on the biological activity in the soil, plant health, as well as on the size and quality of the main plant yield. According to Myśków [1989], the organic matter of the post-crop plants in the form of roots, surface remains, or green mass treated in various ways, influences not only the water and sorptive capacity and the structure of the soil, but also increases the biological activity of that environment. In the presented research, the green mass of the post-crop plants, formed by spring rye and common vetch used as mulch, was left on the soil surface or mixed with the top soil layer through rotary ploughing. The soil from onion cultivation with the mulch of spring rye, regardless of its treatment, contained more (cfu) bacteria than in that with the common vetch mulch. These results confirm prior research, which showed that cereals, including rye, stimulated the growth and development of bacteria [Elliott and Lynch 1984, Solarska 1996, Pięta and Bełkot 2002, Pięta et al. 2002]. According to Pięta and Patkowska [2001], root exudate of winter wheat and rye, as well as the green mass of those plants, had a positive influence also on the development of fungi-saprobionts. Pérez and Ormeño-Nuñez [1991] observed a presence of hydroxamic acids in the root exudates. These acids increase the plants' resistance to pathogen infections [Hartman et al. 1975, Bravo and Lazo 1993]. These acids have an inhibitive effect on the development of cereal soil-borne phytopathogens [Wilkes et al. 1999].

Secondary metabolites, produced by some plant species, when introduced into the soil along with the green mass, may lead to the improvement of the phytosanitary conditions of that environment. These compounds are most frequently produced inside the plant tissue and are released after introducing these plants into the soil after their microbiological decomposition [Gamliel et al. 2000]. Compounds included in the root exudate or released as the result of the green mass decomposition may stimulate or inhibite the development of microorganisms in the soil environment. Among the isolated compounds, the most bacteria- and fungi-static influence was observed in phenols [Funck-Jensen and Hockenhull 1984, Bajus et al. 1986]. The antagonistic *Trichoderma* spp. and *Gliocladium* spp. show high tolerance to the influence of fungi-static substances, as well as antibiotics and enzymes produced by other microorganisms in the soil environment [Papavizas 1985]. Among the bacteria, the highest antagonistic abilities were observed in *Bacillus* spp. and *Pseudomonas* spp. [Fravel 1988, Weller 1988, Defago and Haas 1990, Földes et al. 2000]. The introduction of spring rye as the post-crop plant led to a much higher concentration of the propagation units of the antagonistic bacteria and fungi, than the use of common vetch. The soil from the onion cultivation in combination with spring rye contained much less propagation units of the pathogenic fungi then the combination with common vetch, especially in comparison with the control. It should be suspected that the high concentration of the antagonistic *Bacillus* spp., *Pseudomonas* spp., *Gliocladium* spp. and *Trichoderma* spp. in the soil, led to a substantial reduction of the population of phytopathogens.

The results of the research conducted in the growing chamber showed that the fungi present in the soil proved pathogenic to onion. The agrotechnical method, through an appropriate selection of the post-crop plant, may improve the phytosanitary state of the soil.

CONCLUSIONS

1. Mulch of after-crop plants – spring rye and common vetch have an essential influence on microorganisms composition in soil.
2. Spring rye stimulated the development of microorganisms, especially antagonistic ones, whereas common vetch caused an increase of the number of cfu of pathogens.
3. Fungi from genus *Fusarium culmorum*, *F. oxysporum*, *F. solani*, *Penicillium verrucosum* var. *cyclopium*, P. *verrucosum* var. *verrucosum* and *Pythium irregulare* caused the decrease of numbers and healthiness of onion seedlings.

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WPŁYW MULCZU ROŚLIN POPLONOWYCH I UPRAWY CEBULI NA SKŁAD MIKROORGANIZMÓW W GLEBIE

Streszczenie: Celem badań było określenie składu ilościowego i jakościowego zbiorowisk grzybów i bakterii ukształtowanych w glebie pod wpływem uprawy konserwującej z wykorzystaniem żyta jarego i wyki siewnej jako poplonowych roślin okrywowych. Ponadto w testach laboratoryjnych ustalono występowanie mikroorganizmów wyróżniających się antagonistycznym oddziaływaniem na grzyby patogeniczne o fakultatywnym pasożytnictwie. Wyniki laboratoryjnej analizy mikrobiologicznej wykazały, że poszczególne próby gleby spod uprawy cebuli zawierały różny skład ilościowy i jakościowy zbiorowisk mikroorganizmów. Żyto jare stymulowało wzrost i rozwój mikroorganizmów, szczególnie antagonistycznych (*Bacillus* spp., *Pseudomonas* spp., *Gliocladium* spp., *Trichoderma* spp.), a wyka siewna powodowała wzrost liczby jtk patogenów (*Alternaria alternata, Fusarium culmorum, F. oxysporum, F. solani, Penicillium* spp., *Pythium irregulare*).

Słowa kluczowe: cebula, bakterie antagonistyczne, grzyby antagonistyczne, grzyby patogeniczne, uprawa konserwująca, żyto jare, wyka siewna

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